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## Table of Contents

Title Page	i
Disclaimer	ii
Acknowledgements	iii
Table of Contents	iv
Abstract	1
Executive Summary	1
Report	
a. Introduction	3
b. Results	5
c. Discussion	11
d. Summary and Conclusions	12
References Cited	14
Appendix 1: Release site overview	15
Appendix 2: Evaluation of insectary quality and pre-release conditioning and handling effects using the egg plating method.	16
Appendix 3. Evaluation of environmental effects on egg hatch at 24 hours post-release using the clip cage method in the field.	17
Appendix 4: Evaluation of lacewing survivorship and efficacy against <i>Nasonovia</i> at Bunn2 organic using in-field monitoring after the release.	17
Appendix 5: Pesticide treatments in CCVIPM field release sites.	19
Appendix 6: <i>Nasonovia</i> monitoring in CCVIPM field release sites.	20

## Abstract

A serious new aphid pest in California, *Nasonovia ribis-nigri* (Mosley), has increased applications of broad-spectrum pesticides in Salinas Valley lettuce, threatening developing IPM systems. This project sought to develop an IPM strategy targeting *Nasonovia* in head and leaf lettuce that would include the use of augmentative biological control, specifically green lacewing egg releases, with the use of selective chemical insecticides in an overall insect IPM program for lettuce. To distribute the lacewing eggs, we adapted a liquid electronically controlled delivery system, previously designed and evaluated in laboratory and vineyard tests, for lettuce (row crops). Based on previous work, we conditioned the eggs to be closer to hatch before release and used water as the liquid carrier for the eggs. We completed eight releases in cooperation with three different growers and their Pest Control Advisors (PCAs), six of the releases were conducted in commercial organically-managed lettuce fields. Three of the eight releases failed due to problems with pre-release egg handling or equipment. Each release was evaluated for potential effects on egg hatch and subsequent biological control, including: insectary egg quality, pre-release handling and application effects, environmental effects, lacewing larvae survivorship, and efficacy against the aphid *Nasonovia*. Results showed that lacewing egg hatch of untreated eggs (insectary quality) ranged from 67-93%, while hatch of eggs which had been submersed in water and had traveled through the delivery system ranged from 72-97%, indicating the delivery system did not harm the eggs. However, only one release resulted in lacewing larvae in the field as evaluated by both clip cage and in-field monitoring. In that field, the mean number of lacewing larvae was 1.2 larvae/plant one week after egg application in the release plot compared to 0 in an untreated control plot, and 65% of the treated plants had one or more lacewing larvae. The lacewings did not control the *Nasonovia* population which had a mean of 65 aphids/plant on the same date.

Dislodging of eggs from the plant surface after distribution but prior to hatching appears to be one key factor in poor recovery of lacewing larvae after our egg releases. We conclude that while our liquid electronically controlled delivery system can provide an economical method for safely distributing the eggs, a need exists to identify liquid carriers other than water for distributing the eggs, carriers that improve adhesion of the eggs to the plant without decreasing egg hatch. We will address this objective in our second year of study. If such a carrier can be demonstrated, it is likely that it will improve the recovery of lacewing larvae in the field after an egg release, and enable us to look at the effect of lacewing egg releases for management of *Nasonovia*.

## Executive Summary

*Nasonovia ribis-nigri*, a key pest of lettuce in Europe, is a new aphid pest in California. Because this aphid prefers feeding at the center of the lettuce head, it is extremely difficult to control and infested lettuce is unmarketable. Since it's arrival to California in the fall of 1998, *Nasonovia*'s presence has greatly increased the use of organophosphate insecticides, especially oxydemeton-methyl (Metasystox-R™), on conventionally-grown lettuce. Organic lettuce growers have few, if any, acceptable control options and many have reduced their lettuce acreage because of losses due to *Nasonovia* infestations. In Germany, green lacewings have been shown to be effective biological control agents for *Nasonovia*. This project sought to develop an IPM strategy targeting *Nasonovia* in head

and leaf lettuce that would include the use of augmentative biological control, specifically green lacewing egg releases, with the use of selective chemical insecticides in an overall insect IPM program for lettuce. Specific objectives of the project were: 1.) to evaluate “yield” of green lacewing larvae (*Chrysoperla* sp.) from eggs after discharge from an electronically controlled liquid delivery system and deposition onto field lettuce; and 2.) to evaluate the efficacy of lacewings for control of *Nasonovia* alone and in combination with both selective (IPM) insecticides and standard insecticide programs. Tasks included adaptation of a prototype electronically controlled liquid egg delivery system to lettuce row crops; identification of grower and PCA cooperators for commercial field releases; and development of a methodology to assess lacewing egg and subsequent larvae viability during key points of the release.

We adapted a prototype electronically controlled liquid delivery system, developed and tested for green lacewing egg releases in laboratory and vineyard studies at UC-Davis, (Giles and Wunderlich, 1998) for use in lettuce. The original prototype was expanded from one release valve (similar to a nozzle on conventional spray equipment) to eight valves in order to cover eight seed lines of lettuce with one tractor pass, and an additional vessel (tank) was connected to supply the necessary volume of egg suspension. Based on previous work, we conditioned the lacewing eggs to be closer to hatch before release and used water as the liquid carrier for the eggs. We completed eight releases in cooperation with three different growers and their Pest Control Advisors (PCAs). Because it was difficult to identify conventional growers who were willing to leave portions of their fields untreated, six of the releases were conducted in commercial organically-managed lettuce fields.

Natural enemy releases can fail due to various reasons, including poor insectary quality, improper egg handling, inappropriate release equipment, unfavorable field conditions, and poor survivorship in the field. Three of the eight releases we conducted failed due to problems with pre-release egg handling or equipment. To evaluate Objective 1, final “yield” of lacewing larvae in the field after release with our system, we developed a five-step approach (methodology). Each release was evaluated for potential effects on egg hatch and subsequent biological control at potential points of failure, including: insectary egg quality, pre-release handling and application effects, environmental effects, lacewing larvae survivorship, and efficacy against the aphid *Nasonovia*.

To assess insectary egg quality, three “control plates” of eggs were prepared to determine egg viability in the absence of any experimental effects. Each plate held a maximum of sixty eggs; eggs were separated into cells to prevent cannibalism among hatched larvae. To assess handling and application effects, eggs mixed in water were collected from the distributor valves after travel through the apparatus during each field release and plated for comparison with the control plates. Results showed that lacewing egg hatch of untreated eggs (insectary quality) ranged from 67-93%, while hatch of eggs collected from the distributor ranged from 72-97%, indicating the distributor did not harm the eggs. Since a high percentage of the eggs distributed dislodged, the methodology for assessing egg hatch in the field (environmental effects) was modified to use insect clip cages instead of Stickem Special™ to entrap released eggs and subsequent larvae. To evaluate lacewing

larvae survivorship and efficacy against *Nasonovia*, twenty lettuce plants were monitored weekly following each release in treated and untreated areas of the field. Because only one release resulted in lacewing larvae in the field as evaluated by both clip cage and in-field monitoring, only that release could be evaluated for Objective 2, efficacy of the lacewings against *Nasonovia*. In that field, which was organically-managed, the mean number of lacewing larvae was 1.2 larvae/plant one week after egg application in the release plot compared to 0 in an untreated control plot, and 65% of the treated plants had one or more lacewing larvae. The lacewings, however, did not control the *Nasonovia* population which had a mean of 65 aphids/plant on the same date.

There are several possible reasons why our releases may have failed, including inappropriate weather conditions, inability of first instar larvae to find prey, and dislodging of eggs off of plants before hatching. We conclude that while our liquid mechanical system can provide an economical method for safely distributing the eggs, a need exists to identify liquid carriers other than water for distributing the eggs, carriers that improve adhesion of the eggs to the plant without decreasing egg hatch. We will address this objective in our second year of study. If such a carrier can be demonstrated, it is likely that it will improve the recovery of lacewing larvae in the field after an egg release, and enable us to look at the effect of lacewing egg releases for management of *Nasonovia*.

## **Report**

### **a. Introduction.**

A serious new aphid pest in California, *Nasonovia ribis-nigri* (Mosley), threatens developing IPM systems in Salinas Valley lettuce. Since the aphid prefers feeding at the center of the lettuce head, it is difficult to control and infested lettuce is unmarketable. The aphid's presence in fields has increased applications of broad-spectrum pesticides, especially oxydemeton-methyl (Metasystox-R), and disrupted IPM for other insect pests in lettuce. The goal of this project was to develop an IPM strategy targeting *Nasonovia ribis-nigri* in head and leaf lettuce that would be complementary to an overall insect IPM program for lettuce. The proposed IPM strategy included 1.) the use of augmentative biological control, specifically green lacewing egg releases, with 2.) the use of selective chemical insecticides in 3.) an overall insect IPM program currently being implemented and evaluated by the Central Coast Vegetable IPM Project (CCVIPM). The CCVIPM Project was formed in 1997 with funding from the Pew Charitable Trusts and conducts side-by-side "standard" and "IPM" trials in on-farm commercial lettuce and celery fields.

Since the pest is new to California and the Western U.S., there is no published research data on control here. The volume of published work on *Nasonovia ribis-nigri* originates from researchers in British Columbia and Europe, where it is a major pest in lettuce (Martin, et al., 1996; Mackenzie and Vernon, 1988). Work by Rufingier et al. (1997) and Martin et al. (1996) showed resistance of *Nasonovia* to several insecticides, including endosulfan, methomyl and acephate. In the same study, Martin et al. (1996) found that a single application of imidacloprid prevented aphid infestations establishing on salad crops. Two German studies (Rossmann and Fortmann, 1989; Quentin et. al., 1995) have shown

the effectiveness in using green lacewings for control of *Nasonovia*. Only green lacewings have been reported as effective natural enemies for *Nasonovia* in the European literature we reviewed.

We designed this project to meet the following priority area criteria by developing critical components of a pest management system that is threatened by both regulatory and biological challenges, specifically:

1. Addressing a new pest infestation;
2. Addressing a pest that has demonstrated resistance to several pesticides in Europe;
3. Developing alternatives to the current standard pesticide regime (organophosphates and carbamates) which is under regulatory scrutiny from FQPA and
4. Developing alternatives to the current pesticide regime which poses environmental and human health risks.

This project sought to develop alternatives to the current standard pesticide regime (organophosphates and carbamates) by testing the feasibility of augmentative biological control in commercial scale production using a unique natural enemy delivery system. The mechanization of release techniques can reduce the cost of using biological control and, therefore, make it more cost competitive with standard pesticide practices. Specific objectives of the project were: 1.) to evaluate “yield” of green lacewing larvae (*Chrysoperla* sp.) from eggs after discharge from an electronically controlled liquid delivery system and deposition onto field lettuce; and 2.) to evaluate the efficacy of lacewings for control of *Nasonovia* alone and in combination with both selective (IPM) insecticides and standard insecticide programs.

For Objective 1, to evaluate “yield” of green lacewing larvae (*Chrysoperla* sp.) from eggs after discharge from an electronically controlled liquid delivery system and deposition onto field lettuce, the following tasks were performed:

Task 1a. Adaptation of a prototype electronically controlled liquid egg delivery system to lettuce row crops;

Task 1b. Proper handling and pre-conditioning of commercial green lacewing egg insectary shipments;

Task 1c. Mounting and operation of release equipment, in cooperation with growers, on commercial field sites, and

Task 1d. Development of a methodology to assess lacewing egg and subsequent larvae viability during key points of the release.

For Objective 2, to evaluate the efficacy of lacewings for control of *Nasonovia* alone and in combination with both selective (IPM) insecticides and standard insecticide programs, the following tasks were required:

Task 2a. Identification of conventional grower-cooperators with *Nasonovia* infestations who were willing to leave a portion of their lettuce untreated for lacewing egg releases and who could provide the necessary logistics for commercial field testing;

Task 2b. The successful mechanical application of eggs and survivorship of subsequent lacewing larvae in untreated, selective (IPM) insecticide-treated and standard insecticide treated portions of a commercial lettuce field; and

Task 2c. Monitoring of lacewing and *Nasonovia* populations in untreated, selective (IPM) insecticide-treated and standard insecticide treated portions of a commercial lettuce field that had received the successful application of lacewing eggs using the mechanical delivery system.

## **b. Results**

**Objective1: to evaluate “yield” of green lacewing larvae (*Chrysoperla* sp.) from eggs after discharge from an electronically controlled liquid delivery system and deposition onto field lettuce.**

### Task 1a. Adaptation of a prototype electronically controlled liquid egg delivery system to lettuce row crops.

The first phase of the project consisted of adapting the prototype electronically controlled liquid delivery system developed at UC-Davis (Wunderlich 1997; Giles and Wunderlich, 1998; Wunderlich and Giles 1999) for use in lettuce. The original prototype was expanded from one release valve (similar to a nozzle on conventional spray equipment) to eight valves in order to cover eight seed lines of lettuce with one tractor pass. The valves were mounted onto standard knives welded together to form an inverted “T” which allowed mounting onto any two inch tractor tool bar. Also, an additional vessel (tank) was constructed so that each of the two vessels supplied four valves. An air compressor pressurized each vessel and platforms were constructed for mounting the vessels and compressors to the tractor tool bar. Electrical connectors were wired from each valve to a control box, mounted near the tractor driver, and then to the tractor battery. The control box allowed the frequency and period of the valves to be set and also controlled the power to the air compressors. Thus, the entire lacewing egg delivery system could be dismantled, transported to the field site, and mounted onto any typical tractor tool bar, allowing versatility in bringing the lacewing egg distributor to various grower-cooperators.

### Task 1b. Proper handling and pre-conditioning of commercial green lacewing egg insectary shipments.

For each release, green lacewing eggs, *Chrysoperla rufilabris* (Burmeister), were obtained from a commercial insectary (Beneficial Insectary, Oak Run, CA.). Eggs were packed without carrier and shipped either overnight standard or overnight priority. Upon receipt, three “control plates” of eggs were prepared to determine egg viability in the absence of

any experimental effects, as in Wunderlich and Giles, 1998. Since green lacewing larvae are generalist predators and cannibalistic, the control eggs were separated by placement into individual cells on cell plates and incubated until hatch. Each plate held a maximum of sixty eggs, with a total sample size of approximately 180 eggs/date. Insectary quality data from each release was obtained from the control plates. Control hatch ranged from 67% to 93% for the eight release dates, the overall average was 81% hatch (see Figure 2.1 in Appendix 2).

Wunderlich and Giles (1999) found that the conditioning of eggs by incubation prior to release was an important factor in optimizing yield of larvae from an egg release. Therefore, we conditioned all eggs for release by placing them in an incubator at approximately 30°C (+/- 5°C) until the eggs turned a gray-brown in color, approx. 24- 42 h. The eggs were then removed from the incubator and held at room temperature, approx. 20-22°C, until the morning of the release. Since our incubator does not have a humidifier, humidity was provided by placing a cup of water inside the chamber. *Chrysoperla rufilabris* prefers humid conditions (Tauber and Tauber, 1983) and our incubation chamber may not have provided enough humidity for optimal hatch, contributing to some of the low control hatch (67% on 10/7) we experienced. We have since improved the humidity for pre-conditioning our eggs by placing the eggs and control plates inside closed plastic boxes containing a cup of water and then placing these boxes inside the incubator. We have measured the relative humidity to be 69-75% inside the boxes compared to 30-35% outside the boxes but inside the chamber.

Because releases were dependent on grower's schedules and subject to change, optimizing egg conditioning to time hatch of the larvae with the release was less predictable than expected. We experienced problems with improper pre-release egg conditioning and handling on two release dates (see Figure 2.2 in Appendix 2). For the release on 7/9, most of the eggs for release were completely hatched the morning of the release. Based on that experience, incubation was performed cautiously and eggs were removed from the incubator before the morning of the release. During the release on 8/22, the tractor broke-down in the field during the release and the cooler top was inadvertently left off of the eggs during the time the tractor was repaired in the field. The eggs became too hot and expired, thus the poor hatch from the "mechanical eggs" on that date.

#### Task 1c. Mounting and operation of release equipment, in cooperation with growers, on commercial field sites.

For all of our releases, the electronically controlled liquid delivery system was transported to the field site and mounted on the tractor tool bar the morning of the release. The cooperating growers provided the tractor and the tractor driver for each release, which took up to five hours including mounting, troubleshooting and actual application time. This was a significant contribution of grower's time and resources and required careful logistical planning and communication. One release was cancelled due to an irrigation, and two release sites were changed due to wet field conditions. Table 1 in Appendix 1 gives an overview of all the release sites and lists potential limitations we experienced.

For all of the releases except the first, a mass of 2.7g of eggs was measured into container cups, based on 11,155 eggs/gram and a release concentration of 10 eggs/ml in three liters of water. For the first release, we used a higher concentration of 28 eggs/ml to achieve our desired rate of two eggs/lettuce plant based on the flow rate. This concentration proved too high and the valves became plugged during the release, possibly contributing to a poor hatch in the field. Lowering the concentration to 10 eggs/ml alleviated problems with the distributor plugging, however this meant that the tractor had to make several passes over the same seed line to achieve the high application rates we desired. We are currently working to solve this problem by increasing the droplet size of our egg suspension during application.

Eggs were mixed into the water immediately before loading and distribution in the field. One cup, or approx. 30,000 eggs, was mixed with three liters of water in each of the two vessels. Application rates varied from 30,000 to 120,000 eggs/acre during the releases. For the hand-applied comparison treatment, .063 g of eggs was mixed with 25 ml of water to achieve the concentration used in the mechanical system. The eggs were then “painted” onto leaves using a fine paintbrush.

#### Task 1d. Development of a methodology to assess lacewing egg viability during key points of the release.

Natural enemy releases can fail due to various reasons and there are few guidelines for evaluating releases in a manner that helps us to understand what went wrong when we do not experience the expected results, i.e. efficacy against the target pest. Therefore, we developed a methodology to evaluate each release at potential points of failure, including: insectary egg quality, pre-release handling and application effects, environmental effects, lacewing larvae survivorship, and efficacy against the aphid *Nasonovia*.

For insectary egg quality, the cell plating method previously described under Task 1b was used. To assess handling and application effects, eggs mixed in water were collected from the distributor valves after travel through the apparatus during each field release except the first and plated for comparison with the control plates. Results showed that lacewing egg hatch of untreated eggs (insectary quality) ranged from 67-93% hatch, while hatch of eggs collected from the distributor ranged from 72-97%, indicating the distributor did not harm the eggs (see Figure 2.2 in Appendix 2). Problems with poor pre-release conditioning and handling accounted for the low mechanical hatch on 7/9 and 8/22, as previously noted.

Evaluation of environmental effects, temperature and wind, on egg hatch in the field after distribution proved difficult and required modification. For the first three releases, eggs were visually located on the lettuce leaves after distribution and a circle of nonpoisonous adhesive, (Stickem Special™, Peaceful Valley Farm Supply, Grass Valley, CA), was drawn around each egg to protect it from predation and prevent escape of the hatched larva, as in Wunderlich, 1997. Because of windy conditions and the use of water as a carrier, a large number of circled eggs dislodged into the Stickem Special™ and could not be evaluated for hatch. After the first release, only 24-48% of the eggs remained for assessment, while only 40% remained after the second release and 32% remained after the

third release. Insect clip cages were constructed and used for assessment in the last five releases. The clip cages allowed a greater sample size of eggs to remain for hatch assessment. After the first trial with the cages at Bunn2 on 7/23, 91% of the eggs covered with clip cages remained, and 67-89% of the eggs covered with clip cages remained after the second trial with the cages at Frew110 on 8/22.

Figure 3.1 shows the clip cage results evaluated approximately 24 hours after release at the Cauley 23 field site after three release dates. The figure shows the comparison of hatch mechanically distributed on two varieties, Romaine and Greenleaf, to a painted on by hand comparison on two dates. The graph demonstrates a problem with the clip cage method, that is, it is a “snapshot” in time look at hatch which only gives an indication of whether environmental field conditions, such as temperature, are conducive to egg hatch. Once the clip cages are removed, the egg is either recorded as hatched or not hatched, and the clip cage can not be reattached over the egg if it is not yet hatched. Therefore, Figure 3.1 shows that the hatch of eggs mechanically distributed onto Romaine ranged from 45-57% and those eggs that were hand-placed onto Romaine ranged from 60-53% the day after the release, again demonstrating that the delivery system did not harm the eggs and indicating that environmental conditions appeared conducive to hatch. However, the eggs mechanically distributed onto Greenleaf ranged from 29-62% hatch and those hand-placed ranged from 79-44% the day following the release. We do not believe this indicates a varietal effect, rather it is most likely due to the timing of the evaluation. Still, the clip cage method gives a good indication of whether field temperatures are conducive to lacewing egg hatch and if one can then expect to find lacewing larvae in subsequent field monitoring.

To evaluate lacewing larvae survivorship, and efficacy against the aphid *Nasonovia*, each field was monitored before and after each release for the presence of *Nasonovia* and lacewing larvae. The day before the release and weekly thereafter, twenty plants were monitored in each area of the field to receive eggs and in an untreated area. Lettuce plants were inspected and the number of aphids, apterous and alate, as well as other insect pests and any natural enemies present were counted. Native green lacewings were noted and collected, reared to adults and saved for identification.

Of the eight releases we conducted, three failed due to problems with pre-release egg handling and conditioning or problems with the equipment as previously noted. Of the remaining five releases, only one resulted in good recovery of lacewing larvae in the field following the release. That release occurred on Bunn2, an organically-managed field. On 7/29, one week following the release, 65% of the monitored plants had at least one lacewing larvae. The average number of lacewing larvae/plant on that date was 1.2. On the next two sample dates, 8/3 and 8/9, an untreated (no lacewings released) area of the field was also monitored for comparison. On both of those dates, lacewing larvae continued to be found in the treated area of the field, while no lacewing larvae were found in the untreated area. Figure 4.1 in Appendix 4 shows the mean number of lacewing larvae/plant from the field monitoring conducted at Bunn2.

Lacewing larvae were observed preying on *Nasonovia* in the Bunn2 release field. Lacewing larvae and pupae were collected from this field before harvest, reared to adulthood, and confirmed to be the species *Chrysoperla rufilabris*, which is not native. Populations of *Nasonovia* at Bunn2, were extremely high and were not affected by the lacewing release, however. The week of the first release and the first date the field was monitored, on 7/16, the average number of *Nasonovia*/plant was 15 aphids. By the second week, (and day of the second release which was successful), the population of aphids had already doubled to a mean of 30 aphids/plant. The population continued to increase to a mean of 74 aphids/plant on the last sampling date. The mean number of aphids/plant in the release plots was not different from the mean number of aphids/plant in the untreated plot. Figure 4.2 in Appendix 4 shows the *Nasonovia* field monitoring from Bunn 2.

**Objective 2. To evaluate the efficacy of lacewings for control of *Nasonovia* alone and in combination with both selective (IPM) insecticides and standard insecticide programs.**

Task 2a. Identification of conventional grower-cooperators with *Nasonovia* infestations who were willing to leave a portion of their lettuce untreated for lacewing egg releases and who could provide the necessary logistics for commercial field testing.

We planned to conduct our lacewing release field trials on commercial scale farms with Central Coast Vegetable IPM (CCVIPM) Project cooperators. The CCVIPM Project was formed in 1997 with funding from the Pew Charitable Trusts and conducts side-by-side “standard” and “IPM” trials in on-farm commercial lettuce and celery fields. The “IPM” plots are limited in the scope of pesticide classes used: no organophosphates, carbamates or pyrethroids are allowed when “softer” alternatives exist. Thus, for aphid control, imidacloprid (soil applied Admire™ or foliar Provado™) is the only allowed insecticide on the “IPM” plots in the CCVIPM. We had anticipated the registration of a new novel selective material for sucking insects, pymetrozine (Fulfill™), but Fulfill™ did not receive a registration for lettuce last season and so was not available for use in our field trials. The “standard” side allows all pesticide classes. Each field is monitored weekly by the Project Coordinator and cooperating Pest Control Advisors (PCAs).

1999 was the first full season Salinas Valley growers have had to manage *Nasonovia* as a major new pest. Populations of *Nasonovia* were extremely high and built rapidly in untreated areas, creating a possible source for infestation. Although many growers expressed interest in trying the lacewing releases, few conventional growers were willing to leave any portions of their fields without pesticide treatments for the experimental design. Due to the poor efficacy of imidacloprid in many fields, most CCVIPM cooperators felt it necessary to apply MSR to their IPM plots. Therefore, of the eight releases we conducted, only 2 were on conventional, CCVIPM fields, Frew 110 spring and Frew 110 fall (see Table 1 in Appendix 1). The other six releases were conducted in fields with organic growers who were able to leave large areas of their fields untreated and who also expressed a high level of interest in biological control.

Task 2b. The successful mechanical application of eggs and survivorship of subsequent lacewing larvae in untreated, selective (IPM) insecticide-treated and standard insecticide treated portions of a commercial lettuce field.

Several releases were conducted in CCVIPM fields (Frew 110 spring and Frew 110 fall) with egg applications on untreated, selective IPM insecticide-treated and standard insecticide treated areas. Appendix 5 gives the pesticide treatments for Frew 110 spring and Frew 110 fall. However, due to problems with equipment and handling of eggs, none of these releases resulted in a successful application of eggs with survivorship of lacewing larvae (refer to Table 1 in Appendix 1). The grower went to great lengths of cooperation to leave several beds untreated but was forced to spray this area out when the lacewing releases failed and aphid populations reached intolerable numbers.

Task 2c. Monitoring of lacewing and *Nasonovia* populations in untreated, selective (IPM) insecticide-treated and standard insecticide treated portions of a commercial lettuce field which had received the successful mechanical application of lacewing eggs.

Figure 6.1 in Appendix 6 shows the *Nasonovia* monitoring data from the CCVIPM release field site, Frew 110 spring. Although the untreated plot had a much lower mean number of aphids/plant, 2.5 aphids/plant, than the organic site at Bunn2, it was considered unacceptable to the grower. When the lacewing release on 5/13 was not successful, the grower sprayed out the untreated plot on 5/25. (A second release at this site scheduled for 5/20 was cancelled due to grower irrigation.) Table 5.1 in Appendix 5 shows the pesticide treatments for Frew 110 spring. Figure 6.2 in Appendix 6 shows the aphid monitoring, for *Nasonovia* and other aphid species, from the CCVIPM release field site Frew 110 fall. There were four pesticide treatments in this field: Grower standard with Admire, IPM with Admire, Grower standard without Admire, and Untreated (see Appendix 5.2 for a list of pesticide treatments.) Lacewing eggs were released into each portion of the field on August 21, however, the tractor broke down in the field during the release and the eggs overheated before distribution. On August 26 the mean reached 22 aphids/plant in the Untreated (no pesticides applied) area of the field which the grower left unsprayed specifically for the lacewing releases. When the release on August 21 failed, the grower sprayed out the untreated beds.

Grower and PCA Acceptance

Like all pest control methods, the reduced risk method of pest management we are trying to develop here, that is, the successful distribution of green lacewing eggs using a novel mechanical delivery system, is only acceptable to growers and Pest Control Advisors if it works. That is particularly true for conventional growers who have a lower tolerance for aphid pest presence and have chemical tools available to them. Organic growers are especially interested in augmentative biological control and many of them are buying and distributing green lacewing eggs by hand or with makeshift conventional equipment, but reportedly with mixed results. Every grower and PCA we worked with was extremely interested in our approach, both the mechanical system we tested and the evaluation methodology we used, and was willing to contribute their time, resources, and risk crop damage to participate in our field releases. Their willingness to continue to participate in

this work, which is often logistically difficult, is evidence of the value of this research to them and the likelihood of their acceptance of this technique once it is proven.

### c. Discussion

There are several measures of success for this project. The first measures are those of empirical success in delivering the lacewing eggs to the target plant using the liquid electronically controlled delivery system. These are measured by the ease of use of the equipment without technical difficulties in commercial field operations, and by the five-step methodology for evaluating distributed eggs and subsequent larvae viability that we have developed and outlined in the Results section of this report. As we have stated previously, natural enemy releases can fail for a number of reasons. Three of the eight releases we conducted failed due to problems with pre-release egg handling or equipment. The experience we gained from those releases should help prevent similar problems in the future. Of the remaining five, only one release resulted in lacewing larvae in the field, a key measure of success. Lacewing larvae were observed feeding on *Nasonovia* in this field. Yet, this release did not result in the ultimate measure of success, our goal, which is to manage a key pest using augmentative biological control. We believe if we had been successful with earlier releases in that field, we may have been able to build a lacewing population that may have had some impact on the *Nasonovia*.

There are several possible reasons why our other four releases failed, although we are unable to know the exact reasons, we developed our methodology to be able to rule out key potential points of failure. We know from our data that our insectary quality was generally good and that our delivery system did not harm the eggs. Therefore, we can deduce that the point of failure occurred after the eggs were deposited onto the plants. Once deposited, the eggs need to have appropriate temperatures to hatch. *Nasonovia* survives at much cooler temperatures (as low as 34°F) than lacewings, but releases must be timed to optimal survivorship of the larvae, not necessarily with the population of *Nasonovia*. We recommend users wait until native green lacewings are present in the area, as a sign of appropriate temperature conditions, before attempting to augment with additional eggs. Additionally, first instar lacewing larvae are extremely fragile and need to find prey in a timely manner. Our eggs may have hatched in the field but the larvae may not have survived due to their inability to find suitable prey. This is especially a concern for integrating augmentative biological control into conventionally-managed fields where pest populations are generally much lower than in organic fields. Finally, since we used water as a carrier, eggs may have dislodged off of the plants after distribution and before hatching. Dislodging can occur due to windy conditions or to cultural operations that can disturb the plants. Within 24 hours after two of our releases, both on Cauley 23, the grower needed to enter the field to cultivate (which removed some of our clip cages) and to water. In-field logistics are a challenge to doing any on-farm research and we recommend that growers stay out of the field, if possible, for 24 hours following the release. We plan to investigate the potential use of liquid carriers other than water that may improve adhesion to the plant in the upcoming season.

Another type of measure of success is grower's and PCA's interest and acceptance of this promising reduced-risk pest management tool, which would lead to their adoption if successful. We have already cited cooperation on this project as a measure of user interest and acceptance. Since 1999 was the first year of this work, we did not host a demonstration field day (one is planned for the 2000 growing season). We did, however, present our work at two meetings at which growers and PCAs were the target audience: an annual University Cooperative Extension Entomology Meeting held on December 7, 1999 in Salinas, and the Western Sustainable Agriculture Research and Education (WSARE) meeting held March 7, 2000 in Portland, Oregon. At both of these meetings, growers expressed interest in this project by asking pertinent questions and giving experiential feedback. We conducted a survey during the Salinas meeting that asked respondents to list the top insect pests in lettuce and whether any of the information presented would affect their practices. Of 32 responses, 18 considered *Nasonovia* the top insect pest in lettuce and 7 cited the presentation describing this work for affecting their practices (note this meeting did not specifically target organic growers or their PCAs).

### **Summary and Conclusions.**

Project accomplishments include:

- The adaptation of a prototype liquid electronically controlled delivery system for lacewing eggs developed for use in grapes to commercial field lettuce;
- Demonstration that the delivery system does not harm the eggs, agreeing with the earlier work of Wunderlich and Giles, 1999;
- Development of a five-step methodology for evaluating egg and larvae viability at key points in the release;
- Gaining of valuable experience in pre-release egg handling that will improve our likelihood of success in future releases;
- Identification of a number of grower and PCA cooperators who are willing to contribute their time and resources to continue to work with us in commercial on-farm field trials;
- Demonstration that released eggs can survive in the field and develop into successive instars;
- Gaining of valuable experience in monitoring for distributed lacewing larvae and the target pest, *Nasonovia*;
- Identification of a key factor in the failure of our releases: the need to identify a liquid carrier which will improve adhesion of the eggs to the plant without significantly effecting egg hatch.

It is not surprising that the lacewing larvae had no effect on the *Nasonovia* population in the field in which we were successful with our egg release. The aphid population had reached too high a level before our release date. Had we been able to successfully release eggs earlier, with multiple releases in the same field, we may have built up a high enough lacewing larvae population to see some effect on the aphid. Future releases should be timed earlier in lettuce development, with successive releases executed to build lacewing populations in the field. Although we did not control the *Nasonovia*, released larvae were observed preying on the aphids.

There are several possible reasons why our releases may have failed, including inappropriate weather conditions, inability of first instar larvae to find prey, and dislodging of eggs off of plants before hatching. We believe a key factor in improving recovery of larvae from an egg release is the identification of a liquid carrier, other than water, which will improve adhesion of the eggs to the plant without significantly effecting hatch. We will address this objective in our second year of study. If such a carrier can be demonstrated, it is likely that it will improve the recovery of lacewing larvae in the field after an egg release, and enable us to look at the effect of lacewing egg releases for management of *Nasonovia*.

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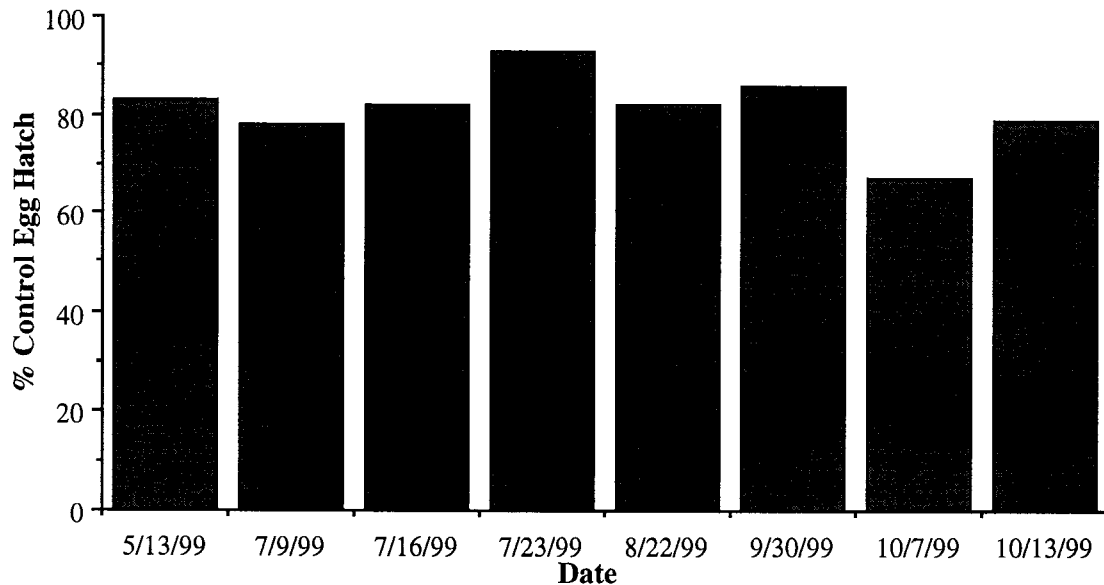
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## Appendix 1. Release site overview.

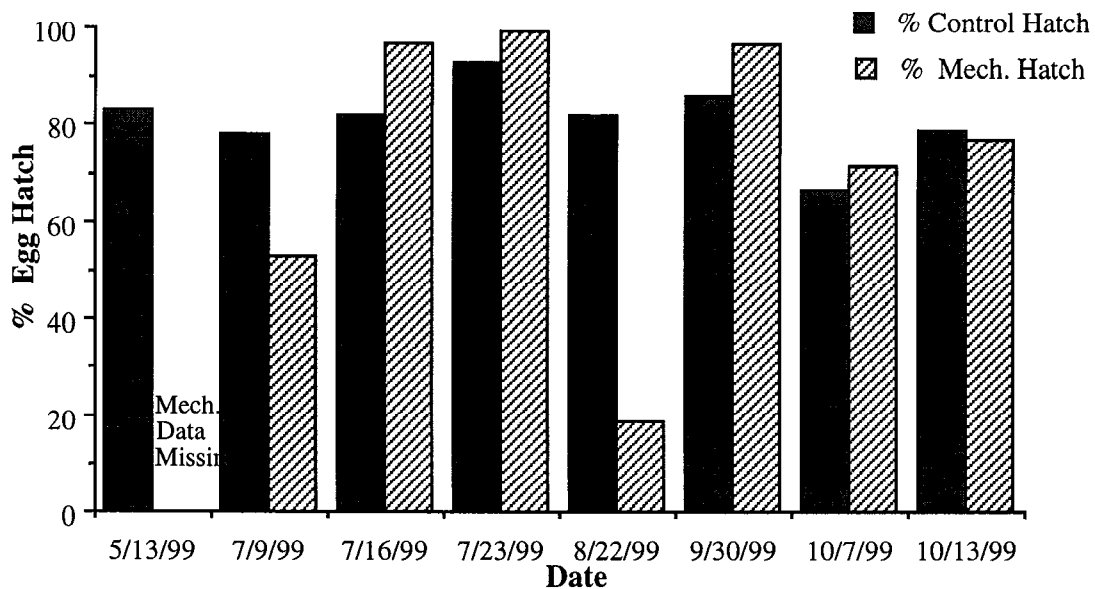
**Table 1:** Lacewing Field Release Attempts and Limitations

<b>Date</b>	<b>Field</b>	<b>Release Completed?</b>	<b>Limitations</b>
5/13/99	Frew 110spring CCVIPM field.	Yes	Eggs at high concentration (28 eggs/ml.) Valves clogged.
5/20/99	Frew 110 2 <sup>nd</sup> release.	No, release cancelled.	Grower irrigation.
7/9/99	Pedrazzi organic	Yes	Release site changed due to grower irrigation schedule. Majority of eggs hatched before release.
7/16/99	Bunn organic	Yes	Release site changed due to wet field conditions. Windy.
7/23/99	Bunn organic, 2 <sup>nd</sup> release.	Yes.	4 of 8 distributor valves malfunctioned due to electrical problem.
8/21/99	Frew 110fall CCVIPM field.	Yes.	Tractor broke down during release. Eggs got too hot while waiting for tractor repair.
9/30/99	Cauley 23 organic.	Yes.	Tractors cultivating after release, knocked off clip cages.
10/7/99	Cauley 23 organic.	Yes.	Hot temps. grower anxious to water after release. Lower insectary hatch than usual.
10/14/99	Cauley 23 organic.	Yes.	Cooler temps. at night.

**Appendix 2: Evaluation of insectary quality and pre-release conditioning and handling effects using the egg plating method.**

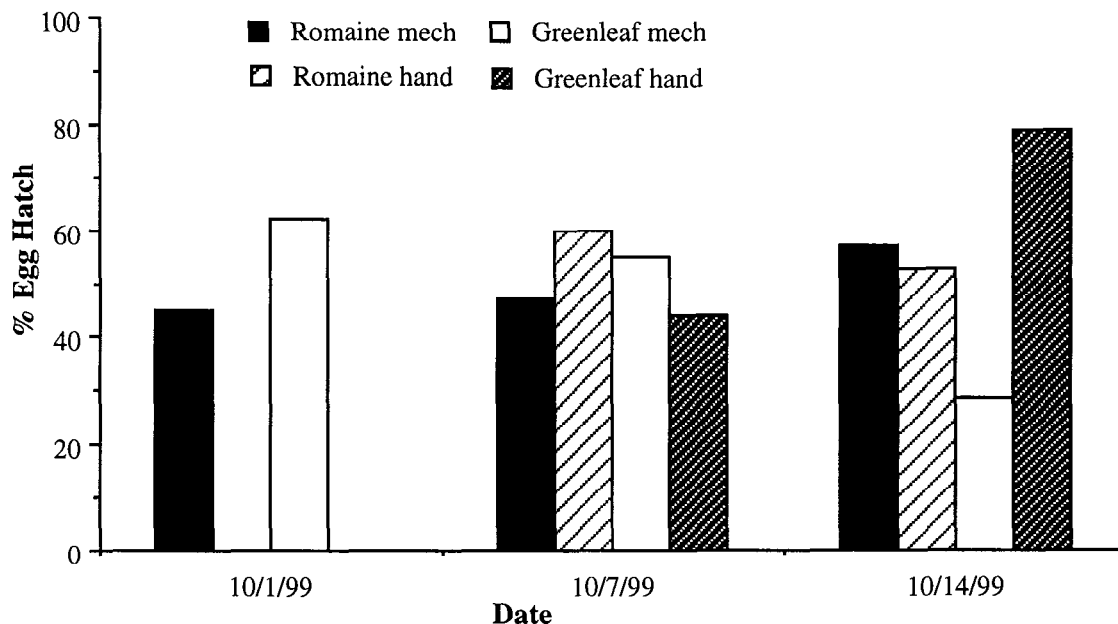


**Figure 2.1** Percent of lacewing egg hatch from untreated, insectary control eggs for each release date.



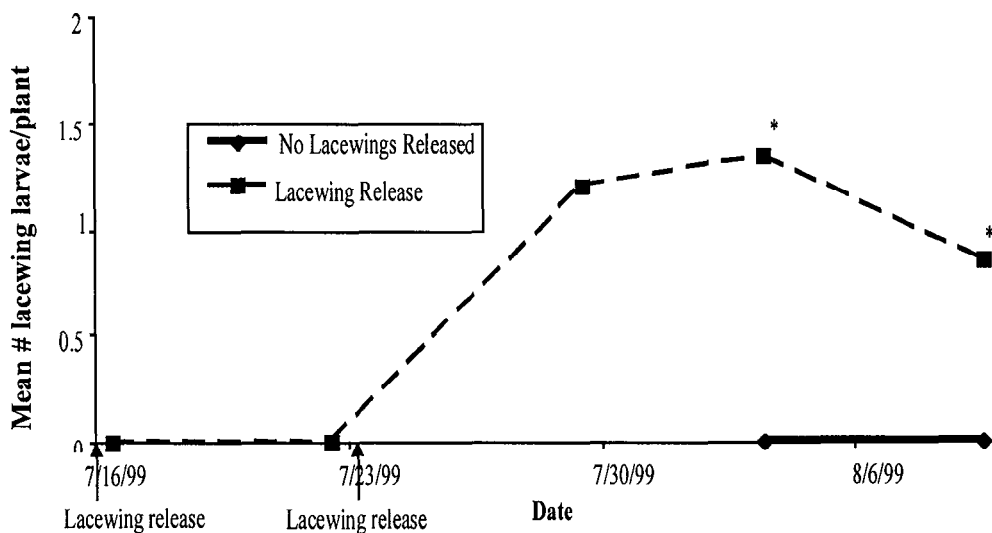
**Figure 2.2.** Percent of lacewing egg hatch from untreated, insectary control eggs and from eggs after submersion in water and travel through the mechanical delivery system for each release date. Problems with pre-release egg handling and delivery equipment occurred on 7/9 and 8/22.

**Appendix 3. Evaluation of environmental effects on egg hatch at 24 hours post-release using the clip cage method in the field.**

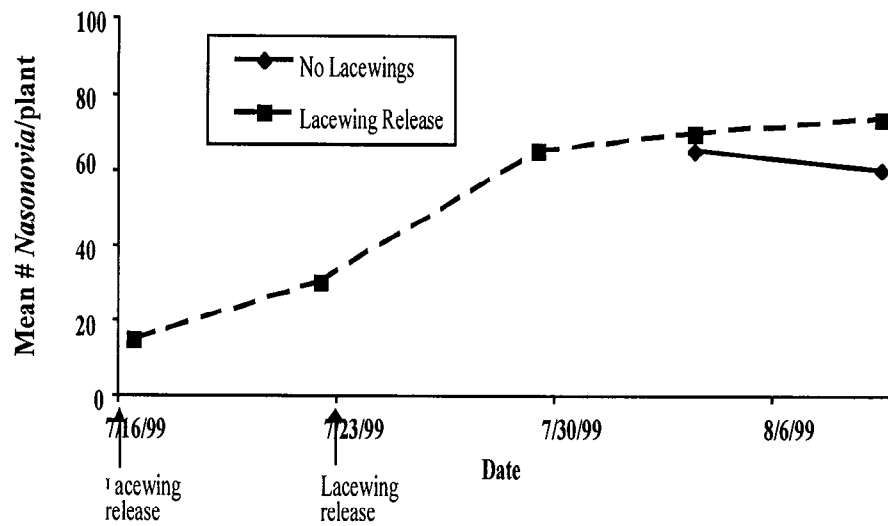


**Figure 3.1.** Percent of egg hatch from eggs enclosed by clip cages approx. 24 hours after release into the field on three release dates. Solid bars indicate eggs distributed mechanically on two different lettuce varieties, Romaine and Greenleaf, and striped bars indicated a painted on by hand comparison.

**Appendix 4: Evaluation of lacewing survivorship and efficacy against *Nasonovia* at Bunn2 organic using in-field monitoring after the release.**



**Figure 4.1.** Mean number of lacewing larvae/plant at Bunn2 organic. Arrows indicate lacewing release dates. An untreated plot (no lacewings released) was monitored 8/3 and 8/9. The lacewing release plot was significantly higher at  $p=0.0001$  than the untreated on both of those dates.



**Figure 4.2.** Mean number of *Nasonovia* aphids/plant at Bunn2 organic. An untreated plot (no lacewings released) was monitored 8/3 and 8/9. There was no significant difference between treatments on either date. Arrows indicate lacewing release dates.

**Appendix 5: Pesticide treatments in CCVIPM field release sites.**

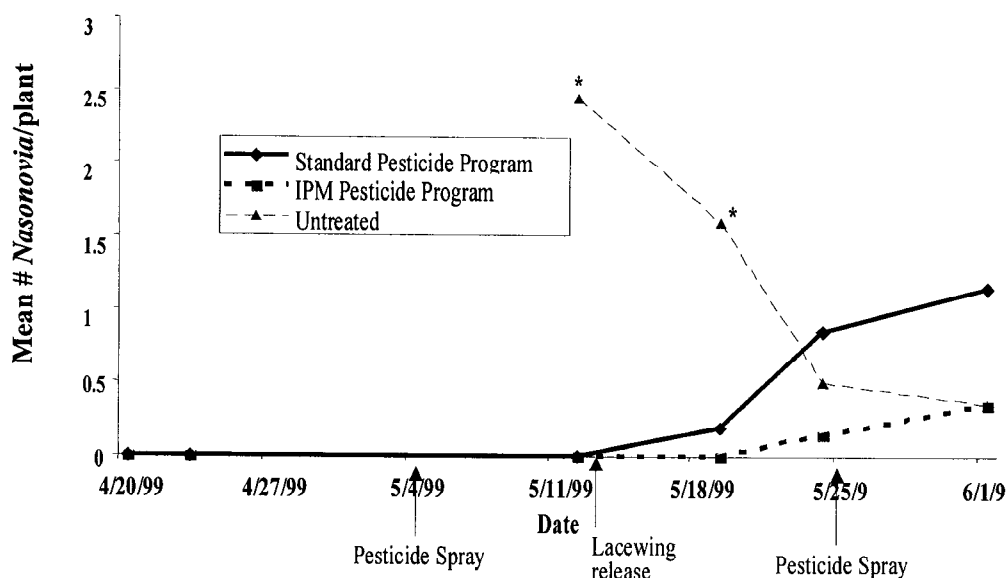
**Table 5.1: Pesticide Treatments at Frew 110 spring.**

<b>Date</b>	<b>Standard rate/ac</b>	<b>IPM rate/ac</b>	<b>Pesticide Untreated rate/ac</b>
<b>05/05/99</b> by ground 75 gal/acre	Provado 1.6 F 3.75 oz. Digon 400 0.5 pts Warrior 3.8 oz Maneb 75 DF 2 lbs.	Provado 1.6 F 3.75 oz. Success 4 oz.  Maneb 75 DF 2 lbs.	
<b>05/25/99</b> by ground 75 gal/acre	Provado 1.6 F 3.75 oz Digon 0.5 pint Warrior 3.8 oz Maneb 75 DF 2 lbs. Sylgard 309 2 oz.	Provado 1.6 F 3.75 oz Success 4 oz.  Maneb 75 DF 2 lbs. Sylgard 2 oz.	Provado 1.6 F 3.75 oz Digon 0.5 pint Warrior 3.8 oz Maneb 75 DF 2 lbs.

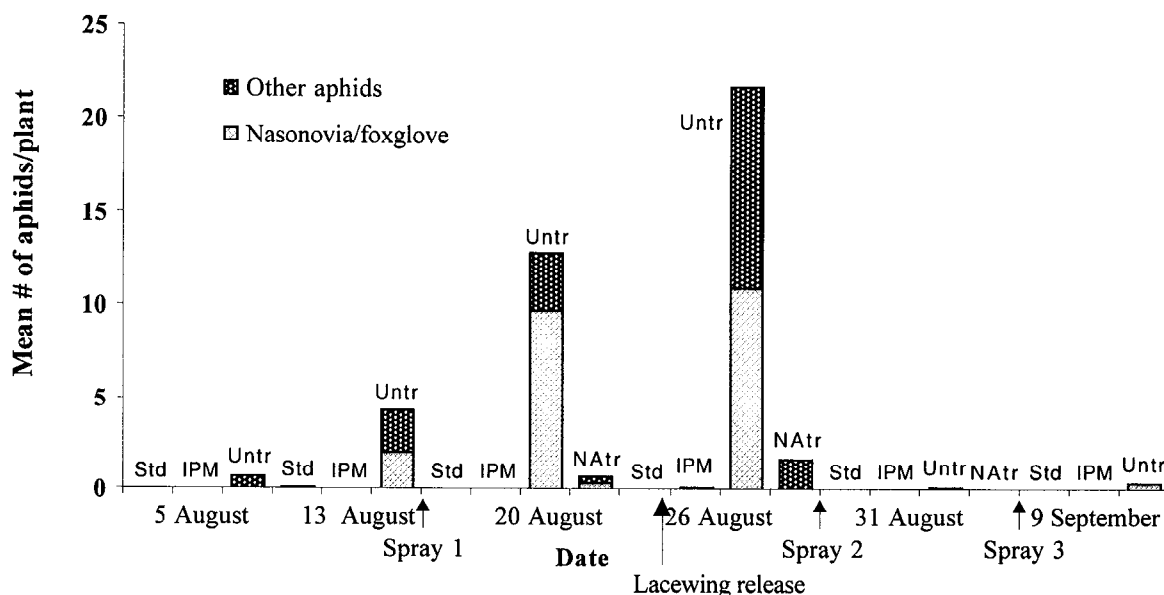
**Table 5.2: Pesticide Treatments at Frew 110 fall.**

<b>Date</b>	<b>Standard rate/ac</b>	<b>IPM rate/ac</b>	<b>“Standard” No Admire 4 beds rate/ac</b>	<b>“Untreated” No Admire 2 beds rate/ac</b>
<b>8 July 1999</b> by ground 30 gal/acre	Admire 20 oz.	Admire 20 oz.		
<b>Spray 1 15 Aug. 1999</b> by ground 50 gal/acre	Digon 8 oz. Maneb 2 lb. R-11 2 oz.	Success 5 oz. Maneb 2 lb. R-11 2 oz.	Diazinon 4 EC 1 pt. Digon 8 oz. Maneb 2 lb. R-11 2 oz.	
<b>Spray 2 27 Aug. 1999</b> by ground 75 gal/acre	Dimethoate 8 oz. Warrior T 3.8 oz Maneb 2 lb. Sylgard 1.5 oz.	Neemix 2 qt. Success 5 oz. Maneb 2 lb.	Provado 1.6F 3.75 oz. Diazinon 4 EC 1 pt. Dimethoate 8 oz. Warrior T 3.8 oz Maneb 2 lb. Sylgard 1.5 oz.	Provado 1.6F 3.75 oz. Diazinon 4 EC 1 pt. Dimethoate 8 oz. Warrior T 3.8 oz Maneb 2 lb. Sylgard 1.5 oz.
<b>Spray 3 5 Sept. 1999</b> by air 20 gal/acre	Success 5 oz. Aliette WDG 3 lb. Potassium carbonate 1.5 lb.	Success 5 oz. Aliette WDG 3 lb. Potassium carbonate 1.5 lb.	Success 5 oz. Aliette WDG 3 lb. Potassium carbonate 1.5 lb.	Success 5 oz. Aliette WDG 3 lb. Potassium carbonate 1.5 lb.

# Appendix 6: *Nasonovia* monitoring in CCVIPM field release sites.



**Figure 6.1:** Mean number of *Nasonovia* aphids/plant at Frew 110 spring. \* indicates treatments are significantly different at  $p = 0.01$ . The lacewing release was conducted on 5/13 and was not successful. A second release scheduled for 5/20 at this site was cancelled due to grower irrigation.



**Figure 6.2:** Mean number of *Nasonovia* and other aphids at Frew 110 fall. There were four pesticide treatments in this field: Grower standard with Admire, IPM with Admire, Grower standard without Admire, and Untreated (see Appendix 5.2 for a list of pesticide treatments.) Lacewing eggs were released into each portion of the field on August 21, however, the release failed due to overheating of the eggs before distribution.